Silica@zirconia core@shell ceramics as vaccine nanocarriers

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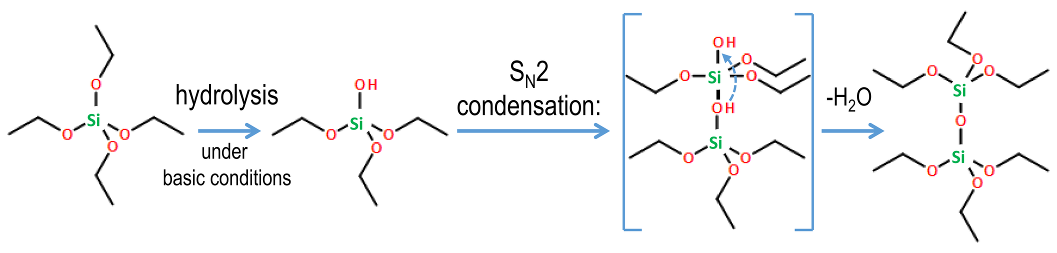
INTRODUCTION

CpG oligonucleotide (ODN) immune stimulators used in cancer immunotherapy were chosen to be adsorbed at the surface of silica@zirconia oxide nanoparticles. The size of the nanoparticles was varied in the size range that is specific for bacteria (20-200 nm). The structure, morphology, colloidal and chemical stability of the native and adjuvant-modified nanoparticles were subject of thorough physical-chemical characterization. After optimization of the structure, the nanoparticles will be added to dendritic cells for toxicity, cell uptake and cell activity experiments. The purpose of this study is to find a size-related effect of the nanocarrier on the obtained immune response.

EXPERIMENTAL

SiO_2 synthesis

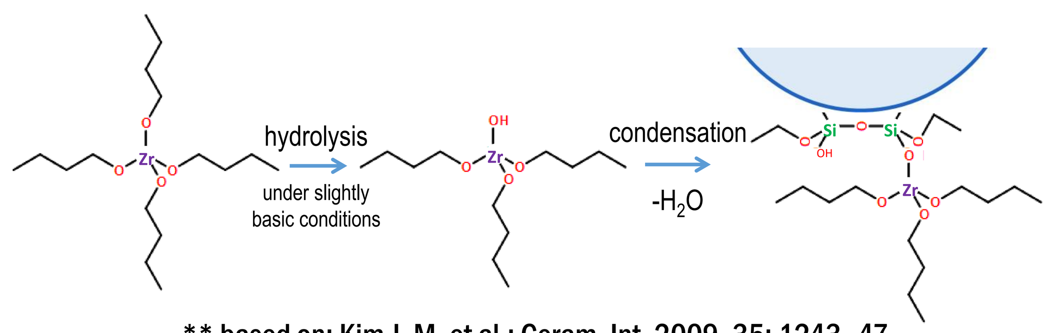
Synthesis of Stöber silica particles of different sizes in ethanol*



*Stöber W., Fink A. and Bohn E.: J. Colloid Interface Sci. 1968, 26: 62-69.

ZrO_2 deposition

Dropwise addition of Zr(IV) tetrabutoxide solution to the diluted silica sol; 50°C; ethanol; argon athmosphere**



** based on: Kim J. M. et al.: Ceram. Int. 2009, 35: 1243–47.

Functionalization

Single stranded synthetic DNAs used in this study:

Immune stimulator 1: CpG ODN 2395

5'-tcgtcgttttcggcgc:gcgccg-3'

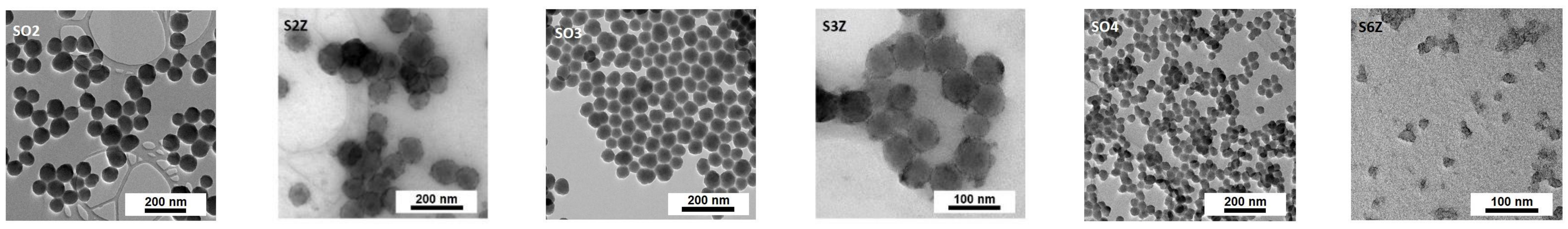
Immune stimulator 2: CpG ODN 1826

5'-tccatgacgttcctgacgtt-3'

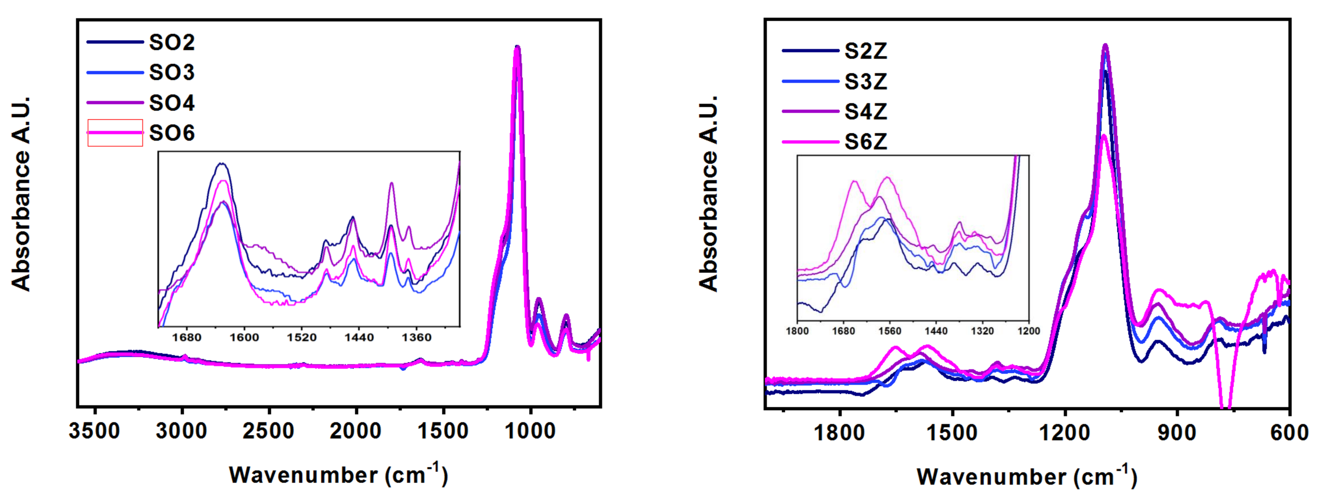
RESULTS – native oxide nanoparticles

Sample	SiO_2 volume mean diameter (nm)	$SiO_2@ZrO_2$ volume mean diameter (nm)	$SiO_2@ZrO_2$ PdI
S2Z	123 ± 34	109 ± 29	0.021
S3Z	75 ± 25	134 ± 53	0.133
S4Z	35 ± 13	47 ± 13	0.043
S6Z	6 ± 3	25 ± 6	0.021

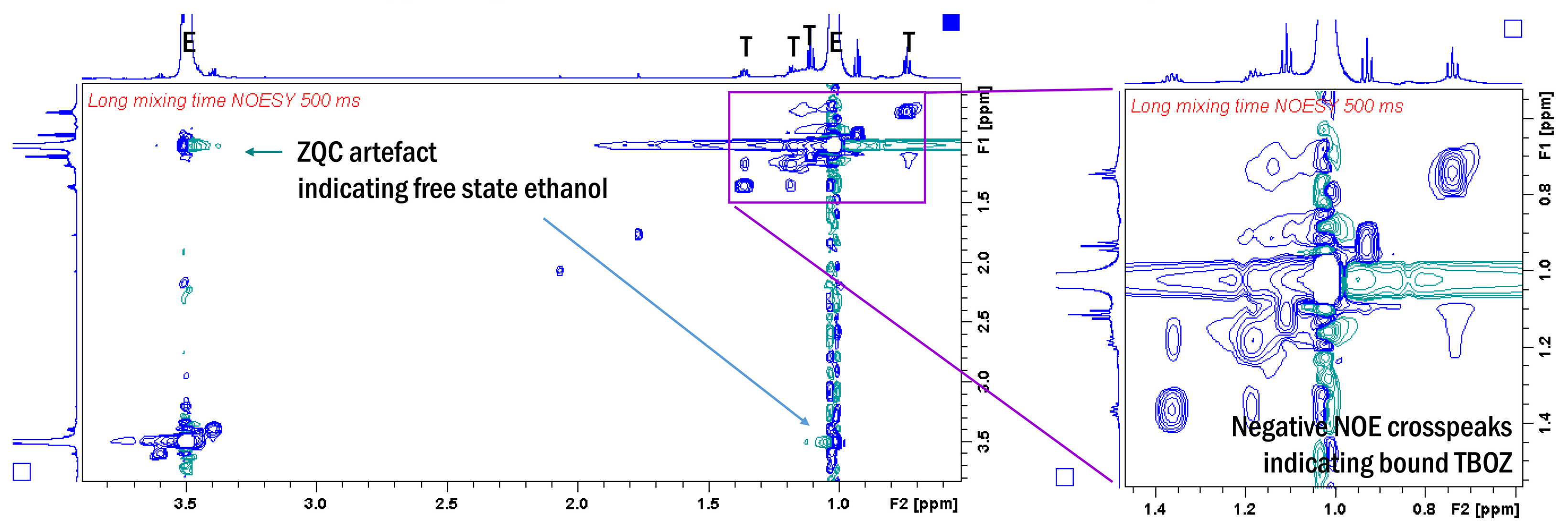
Particle size distribution characteristics derived from DLS



Transmission electron microscopy images of native silica and silica@zirconia samples

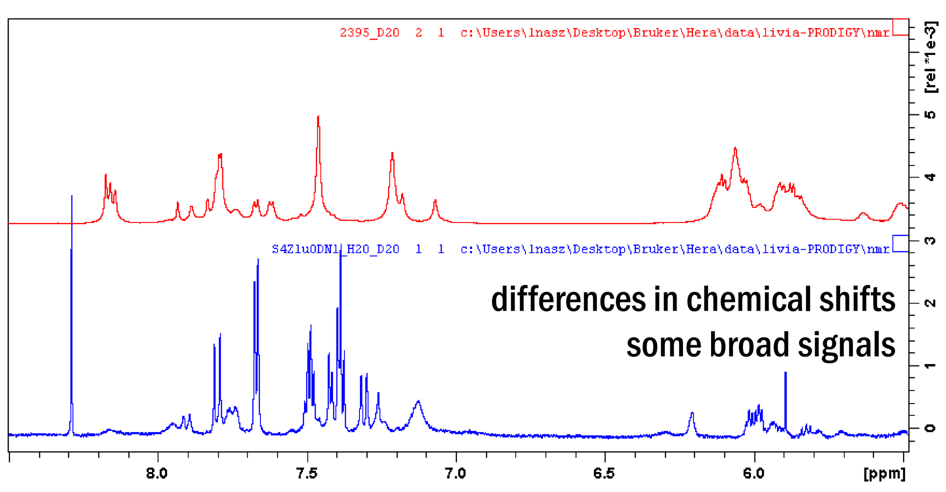


Fingerprint region of infrared spectra for SiO_2 (left) and $SiO_2@ZrO_2$ (right) particles

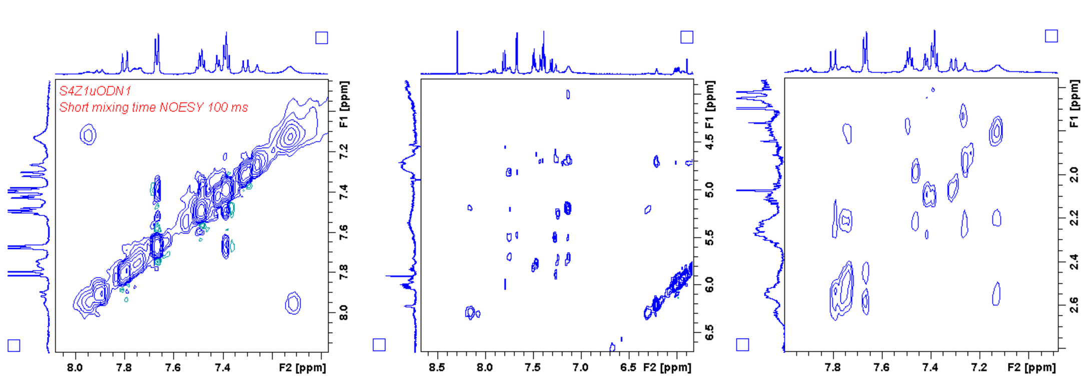


Details of [1]H-[1]H NOESY NMR spectrum showing free state ethanol (left) and surface-interacting TBOZ (right)

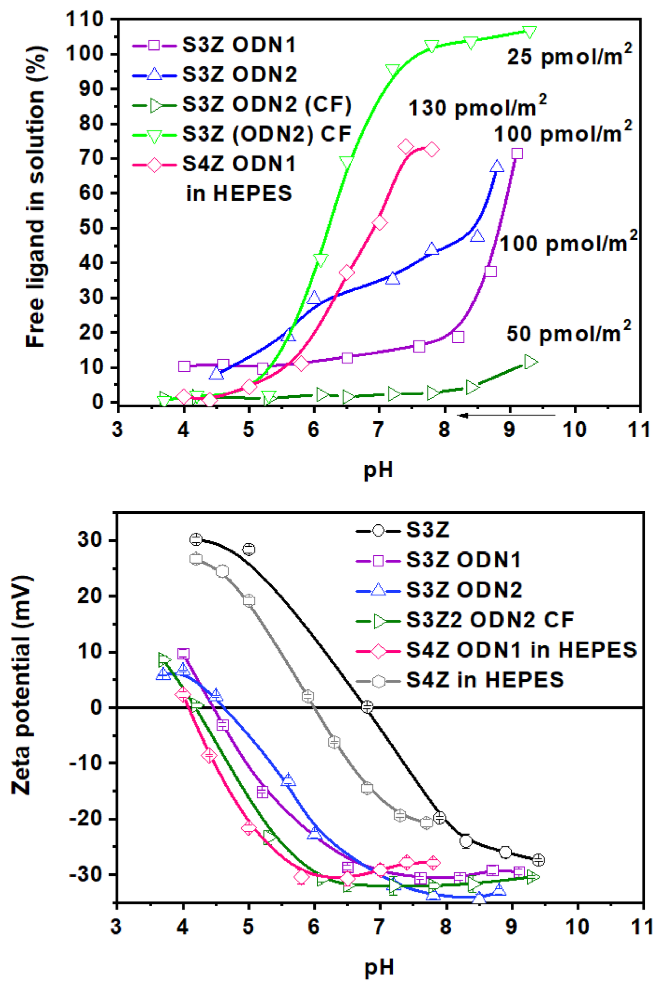
immune stimulator adsorption



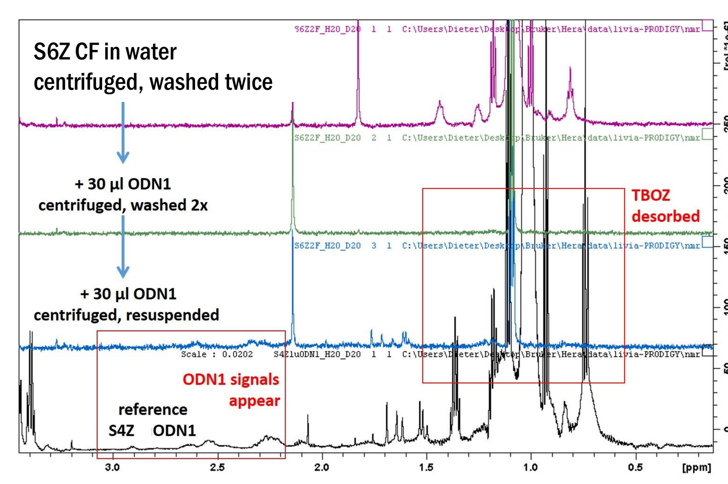
[1]H NMR spectra of ODN1 (red) and S4Z ODN1 (blue)



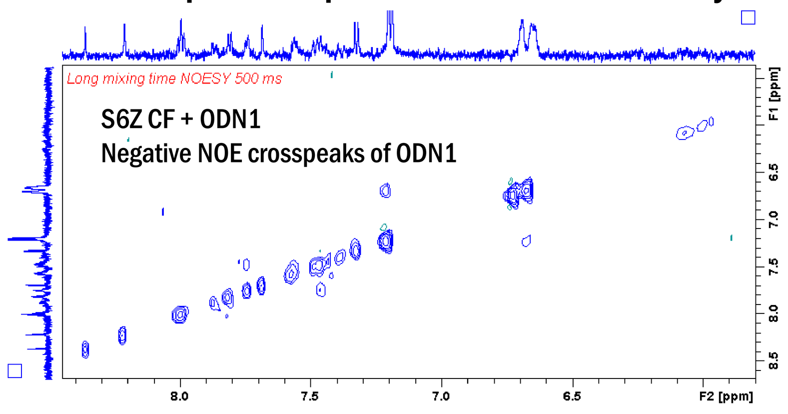
[1]H-[1]H NOESY NMR details of S4Z ODN1: strong negative NOEs reveal binding of ODN1 to S4Z surface



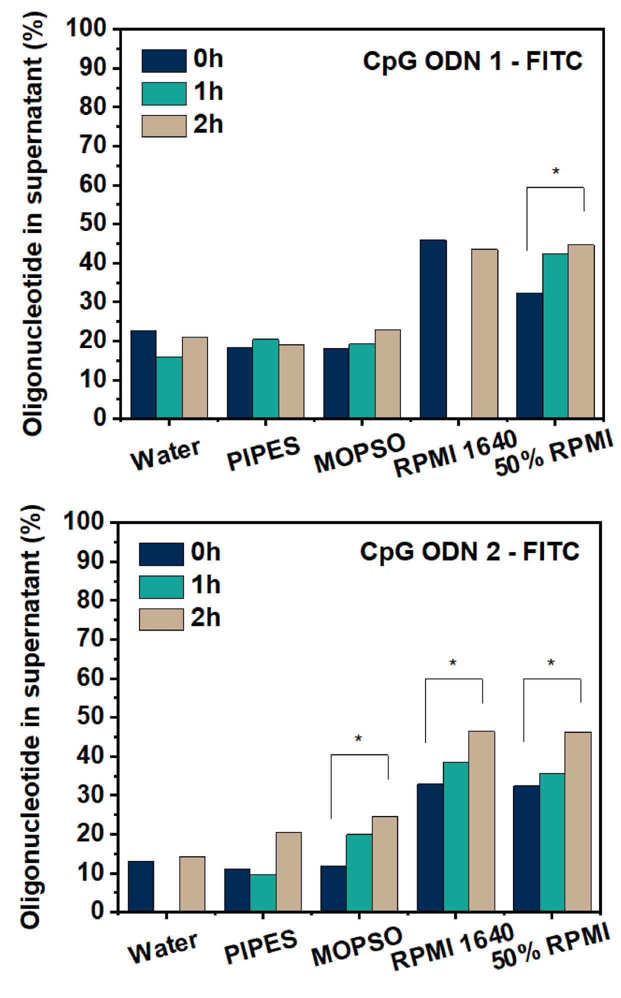
Shifted PZC: the ODNs chemisorb on the surface of NPs (down); the free ligand ratio determined by UV-vis at each pH (up)



Competition of ODN and carboxyfluorescein (CF): at neutral pH CF is pushed off the surface by ODN.



[1]H-[1]H NOESY NMR of S6Z ODN1: negative NOEs reveal binding of ODN1, not CF



Fluorimetry: the ratio of desorbed fluorescently labeled ODNs is monitored from the supernatant in different media

CONCLUSIONS

- The chosen immune stimulators were successfully adsorbed on the surface of different size $SiO_2@ZrO_2$ NPs. The conditions of adsorption were elucidated.
- The effect of buffers on ODN adsorption are currently studied, because HEPES interacts with the nanoparticles (new absorbance peak appearing in UV-vis spectrum). The buffers in focus are phosphate, HEPES, PIPES, MES, MOPS and MOPSO.
- The simultaneous adsorption of 5(6)-carboxyfluorescein and ODNs is not possible at physiological pH because of their competition. Fluorescently labeled ODNs should be used for biological experiments.
- Fluorescently labeled ODNs do not desorb from the NP surface in PIPES and water within 2 hours. However, a slow desorption can be observed in MOPSO, RPMI 1640 cell medium and diluted RPMI 1640 cell medium.

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